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TISSUE CHLOROQUINE LEVELS IN DIABETIC RATS

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ABSTRACT

Diabetes mellitus is a disorder of metabolism resulting from insulin abnormality. Tissue levels of chloroquine in diabetes were investigated. Few diseases have such plural effects in the body as diabetes mellitus. Chloroquine distribution in the tissue and blood was investigated in diabetic rats to a certain how diabetes mellitus affects the levels of chloroquine in tissues and blood. This may prove valuable in a possible diabetes- malaria case treatment. Diabetes mellitus was induced in a group of experimental rats using streptozotocin, 60mg/Kg dose. The mean volume of urine passed over 96 hour period after 10 days of streptozotocin administration was 31.74±0.06 ml and that of control rats was 23.28±0.19 ml. The values showed significant statistical difference ($p<0.05$). Confirmation of diabetes was performed 14 days after streptozotocin administration by determining the blood glucose levels, using glucose oxidase reagent. The diabetic and the control rats were divided into two groups each, which were given 10mg/Kg and 20mg/Kg single dose of chloroquine respectively. 96 hours after the administration of a single dose of chloroquine, the blood and tissue levels of chloroquine were determined. The chloroquine levels obtained in the tissues of the diabetic rats were 1.01±0.06 μgg^{-1}in the heart and 10.73±0.185 μgg^{-1} in the liver for the 10mg/kg single chloroquine dose and 1.68±0.06μgg^{-1} in the heart and 16.68±1.056μgg^{-1}in the liver for the 20mg/kg single chloroquine dose. The corresponding values for the control rats were 0.96±0.032μgg^{-1} in the heart and 7.23±0.072μgg^{-1} in the liver for the 10mg/kg single chloroquine dose and 1.59±0.039μgg^{-1} in the heart and 12.66±0.396μgg^{-1} in liver for the 20mg/kg single chloroquine dose. The diabetic and control rats chloroquine tissue and blood values showed significant statistical difference ($p<0.05$).

INTRODUCTION: Diabetes mellitus is a disorder of metabolism resulting from insufficient production of or reduced sensitivity to insulin. It is a state of chronic hyperglycemia which is responsible for the symptoms and complications of the disease.[1, 2, 3] There are different forms of diabetes mellitus arising from different causes. Type I diabetes mellitus (insulinopenic) arises from the lack of insulin in the body due to the inability of the pancreas to secrete or produce enough insulin needed to mop up blood glucose for storage, energy utilization and synthesis of macro-molecules. It is irreversible and has about 15 % of occurrence. Type II diabetes mellitus (insulinoplethoric) arises from lack of or reduced sensitivity to insulin; more insulin than required is present in the system. It has about 75% of occurrence of diabetes mellitus. Hyperinsulinism or insulin resistance may be correlated with a decrease in insulin receptors, reduced insulin binding or defects in post receptor binding [1, 2, 3].

Recovery from diabetes mellitus never occurs without treatment [1, 3]. It is best managed through dietary intake. If untreated it leads to a buildup of toxic products of impaired metabolism. About 125 million persons worldwide have diabetes mellitus. This figure is expected to reach 220 million by 2010 [4]. Insulin inhibits hepatic glucose production and stimulates the uptake and metabolism of glucose by muscles and adipose tissues. At 20mg/ml, the production of glucose is inhibited half maximally. At 50mg/ml, glucose utilization is stimulated half maximally [5]. Insulin is an inducer of drug metabolism, and lack of this hormone (and/ or lack of quality hormone) may lead to reduced capacity of the liver to metabolize drugs [6]. Insulin misnomer, which is diabetes mellitus, predisposes the body to a lot of diseases like viral, bacterial and parasitic infections. These infections, which activate the immune system, cause a decrease in drug metabolism [7, 8].

The antimalarial drug, chloroquine, is extensively distributed in tissues and slowly eliminated such that after a single dose, a plasma half life of 3- 5 days has been recorded [9, 10, 11]. Chloroquine, a 7-chloro- 4- (- 4- diethylamino- 1- methylamino) quinoline, is usually formulated as the phosphate, hydrochloride or sulphate. These salts, which are white crystalline powder, are soluble in water but insoluble in organic solvents. Chloroquine base when liberated from the salt in alkaline solutions can be extracted by organic solvents [11, 12]. The 4-aminoquinoline is a large molecule in the chloroquine structure, which constitutes the chromophore for light absorption.

Studies have revealed that the renal clearance is about half its total systemic clearance. Unchanged chloroquine is over 50% and the metabolites about 25% urinary products. Studies on autopsy materials revealed the order of decreasing concentration in the liver, spleen, kidney, heart and brain [14, 15, 16]. Chloroquine is metabolized in the body producing desethylchloroquine, bisdesethylchloroquine, 4- hydroxylchloroquine, 4-aminoquino- 7- chloroquine, 4- carboxyderivative, mono- N- oxide and di-N-oxide derivatives as the major metabolites. The use of appropriate buffers has been found to separate chloroquine, the parent drug, from the metabolites [17].

MATERIALS AND METHODS: Albino rats of wistar strain, 160 to 200g were procured from the Animal house of the Department of Pharmacology, University of Benin. These rats were fed on standard diet, weighed and left to acclimatize for 14 days, during which they showed a steady increase in body weight indicating normal heath condition. Diabetes mellitus was induced in a group of rats using streptozotocin (60mg/Kg dose) through intravenous route via the tail vein. Prior to the administration of streptozotocin, the blood glucose levels of these rats were determined. After the administration of streptozotocin, these rats were

monitored alongside the control rats for weight changes, urinary volume, water intake and level of activity. After 14 days, these rats were anaesthetized with chloroform. Blood samples were withdrawn by cardiac puncture into fluoride oxalate tubes. The blood glucose levels of the streptozotocin-treated rats and those of the control rats was analyzed using glucose oxidase reagent. The clear difference and the high levels of glucose in the blood of the streptozotocin-treated rats compared to the earlier determined blood glucose levels and those of the control rats confirmed diabetes. This was in addition to the higher volume of urine recorded for streptozotocin-treated rats, which indicated the induction of diabetes mellitus.

The control and diabetic rats were divided into two sub groups, which were each given 10mg/Kg and 20mg/Kg single doses of chloroquine respectively. After 96 hours, the rats were bled and sacrificed. The heart, liver, kidney, spleen, lungs, plasma and red blood cell samples were collected and analyzed for chloroquine content. The tissues were retrieved, blotted dry and weighed. About 0.5g each was taken and homogenized in 5ml of 0.1M HCl. This was centrifuged and the supernatant layer taken for analysis. Blood samples were placed in fluoride oxalate tubes and immediately spun in a centrifuge for about 10 minutes. The plasma was collected for analysis. 0.5g of the red blood cell residues was homogenized in5ml 0.1M HCl and centrifuged. The supernatant layer was taken for analysis.

Drug Analysis: 2ml each of the samples was placed in a 50ml separating funnel. 0.5ml of ammonia solution (s.g. 0.91) was added and then extracted with 10ml (x2) of diethyl ether by shaking for 2 minutes [18]. The ether extract was bulked and washed with 10ml of borate buffer (pH 9.5) and the ether layer collected. Analysis of chloroquine was done by shaking 15ml of the ether layer with 10ml of phosphate borate buffer (pH 7.85) for 5minutes to remove the metabolites. 10ml of the organic layer was shaken with 10ml of 0.1M HCl for 5 minutes. Then 2ml aliquot from the 0.1M HCl extract was placed in a 50ml separating funnel. 3ml cobalt thiocyanate reagent was added. The solution was equilibrated with 10ml of nitrobenzene by shaking for 2 minutes. The nitrobenzene layer was clarified after separation by centrifugation at 2500 rev. $min.^{-1}$ for 15 minutes. This was filtered into a 10ml flask through a nitrobenzene moistened filter paper and adjusted to 10ml with the later solvent. The absorbance of the solution was measured at 625nm against a blank (2ml of 0.1M HCl instead of the 2ml aliquot from the 0.1M HCl extract). The level of chloroquine was calculated from the calibration curve prepared in the same manner.

RESULTS AND DISCUSSION: The streptozotocin-treated (diabetized) rats, when monitored alongside control (undiabetzed) rats, showed a general loss of body weight, increase in urinary volume, increase in water intake, and lack of vibrancy. The blood glucose level test showed that those of diabetic rats were statistically higher than the control (undiabetzed) rats ($p<0.05$). This confirmed the induction of diabetes mellitus.

The values, in the tables below, are levels of chloroquine estimated from the liver, spleen, lungs, kidney, heart, red blood cells and plasma; for the 10mg/Kg single chloroquine dose (control and diabetic rats) (**table 1**) and 20mg/Kg single chloroquine dose (**table 2**) (control and diabetic rats) respectively. There was a significant statistical difference ($p<0.05$) between the levels of chloroquine estimated in the control and diabetic rats , at both dose treatment. More chloroquine was estimated in the diabetic rats than in the control rats. This signifies that chloroquine is probably less metabolized in the diabetic rats than in the control (undiabetzed) rats [7, 8]. Insulin, the hormone missing in some forms of diabetes mellitus, has been implicated as an inducer of drug

metabolism [1, 2]. Furthermore, immune response in disease states varies. Immune response in disease state tends to reduce the livers ability to metabolize drugs. The levels of chloroquine estimated in the high- perfused tissues and blood agree with what has been reported by other workers, [14, 19, 20] which indicated that the order of decreasing levels in high-perfused tissues is liver, spleen, lungs and kidney. Low levels have been recorded in plasma.

TABLE 1: CONCENTRATION OF CHLOROQUINE IN TISSUES AND BLOOD- 10MG/KG SINGLE DOSE TREATMENT

TISSUE	CONTROL RATS (µg/g±SEM)	DIABETIC RATS (µg/g±SEM)
Liver	7.23±0.072	10.73±0.185
Spleen	7.10±0.047	8.41±0.191
Lungs	5.78±0.082	6.02±0.172
Kidney	4.01±0.156	4.69±0.040
Heart	0.96±0.032 µg/ml	1.11±0.039 µg/ml
RBC	1.11±0.039	1.12±0.039
Plasma	0.059±0.008	0.067±0.005

TABLE 2: CONCENTRATION OF CHLOROQUINE IN TISSUES AND BLOOD- 20MG/KG SINGLE DOSE TREATMENT

TISSUE	CONTROL RATS (µg/g±SEM)	DIABETIC RATS (µg/g±SEM)
Liver	12.66±0.396	16.68±1.056
Spleen	11.40±0.284	12.29±0.184
Lungs	9.16±0.176	9.29±0.489
Kidney	6.12±0.117	6.39±0.048
Heart	1.59±0.032 µg/ml	1.68±0.060 µg/ml
RBC	1.45±0.039	1.58±0.071
Plasma	0.094±0.008	0.094±0.005

CONCLUSION: The estimation of higher levels of chloroquine in diabetic rats is probably due to impairment of metabolizing enzymes or slow metabolism occasioned by diabetes. Such impairment of metabolism due to the malfunctioning of the enzymes required for metabolism of drugs has been previously reported [13]. This study has shown that diabetes has a significant effect on chloroquine levels in urine. It has been observes for the first time that more chloroquine abound in urine in diabetic state.

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